HUMAN FAM19A1/TAA1 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN FAM19A1 CONCENTRATIONS IN SERUM, EDTA PLASMA AND CSF

ALWAYS REFER TO LOT SPECIFIC PROTOCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ AND CHECK ALL ITEMS OF EACH KIT BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PRODUCT INFORMATION:
THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**HUMAN FAM19A1 ELISA KIT**
Catalog No.	SK00419-01
Lot No.	
Formulation	96 T
Standard range	0.5 - 32000 pg/ml
Sensitivity	100 pg/ml
Sample require	100 µl
Dilution Factor	***Optimal dilutions should be determined by each laboratory for each application***
Sample Type	Serum, EDTA Plasma, CSF
Specificity	Human FAM19A1
Calibration	Human FAM19A His Tag Recombinant
Intra-assay Precision	2 - 5%
Inter-assay Precision	4 - 8%
Storage	2 - 8°C for 6 months. See page 3 for detail
This kit contains sufficient materials to run 35-40 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human FAM19A1/TAFA1 ELISA Kit contains the necessary components required for the quantitative measurement of human FAM19A1 from serum, EDTA plasma and CSF in a sandwich ELISA format.

This immunoassay contains human FAM19A1 animal free and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural FAM19A1 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human FM19A1. The capture antibody can bind to the human FAM19A1 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against FAM19A1 is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of human FAM19A1 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
FAM19A1 Microplate - 96 well polystyrene microplate coated with a purified antibody against FAM19A1.	**419-01-01**	**1 plate**
FAM19A1 Standard – 128 ng/vial of human FAM19A1 in a buffered protein base with preservative; lyophilized.	**419-01-02**	**1 vial**
Detection Antibody – 1.2 mL/vial, 10-fold concentrate of a biotinylated antibody against FAM19A1 with preservative; lyophilized.	**491-01-03**	**1 vial**
Streptavidin HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Streptavidin-HRP conjugate.	**SAHRP**	**1 vial**
Dilution Buffer – 45 mL and 12 mL of buffered protein based solution with preservative.	**DB10**	**1 bottle**
Antibody Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB108C**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB08B**	**1 bottle**
Wash Buffer - 25 mL of 20-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution -11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.25M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 - 8°C for up to 6 months. For longer storage up to 10 months, unopened Standard, Positive Control and Detection Antibody Concentrate, Diluent Buffer and HRP Diluent Solution should be stored at -20°C. Streptavidin HRP Conjugate and TMB Substrate Solution should be stored only at 2 ~ 8 ° C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (200 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

EDTA Plasma samples may require 100 - 200 fold dilution.
A suggested 50 -fold dilution is 5 µL sample + 245 µL **Dilution Buffer (DB10)**. A suggested 10 -fold dilution is 50 µL per well of 50-fold diluted sample + 50 µL per well of **Dilution Buffer (DB10)**.

Optimal dilutions should be determined by each laboratory for each application. It is very important to pretest the sample dilution before performing the final assay.
Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

FAM19A1 Standard - Reconstitute the **FAM19A1 standard** with 1.0 mL of **Dilution Buffer DB10**. This reconstitution produces a stock solution of 128 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of **Dilution Buffer (DB10)** into tubes #2 to #7. Use the stock solution (20 ng/mL) to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **32000 pg/mL** standard serves as the high standard. **The Dilution Buffer (DB10)** serves as the zero standard (0 pg/mL). Store the stock solution at -70°C for a few days.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1.0 mL	128 ng/ml
# 1	150µl of stock	450µl	32000 pg/ml
# 2	250µl of 1	250µl	16000 pg/ml
# 3	250µl of 2	250µl	8000 pg/ml
# 4	250µl of 3	250µl	4000 pg/ml
# 5	250µl of 4	250µl	2000 pg/ml
# 6	250µl of 5	250µl	1000 pg/ml
# 7	250µl of 6	250µl	500 pg/ml

Detection Antibody - Reconstitute the Detection Antibody Concentrate with **1.2 mL** of **Dilution Buffer (DB108C)** to produce a 10-fold concentrated stock solution. For 96 test, freshly Transfer 1.2 mL of 10-fold concentrated stock solution to 10.8 mL of **Dilution Buffer (DB10)** to prepare working solution.

For partial strips test, freshly prepare 900 µl per strip (8-well) of working solution. Store the 10-fold concentrated stock solution at -20 °C for a few days.

Streptavidin-HRP Conjugate - For 96 test, freshly Transfer 110 µl of 100-fold concentrated Streptavidin-HRP conjugate stock solution to 10.89 mL of **HRP Diluent Solution (DB08B)** to prepare working solution ***(protect from light)****. The working solution of Streptavidin-HRP Conjugate should be freshly prepared and used within 10~ 20 minutes.*

For partial strips test, freshly prepare 900 µl per strip (8-well) of working solution. Store the 100-fold concentrated stock solution at 2 ~ 8 °C for 10 months.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 µL per well of Dilution Buffer (**DB10**) to Blank wells.
3. Add 100 µL of Standard solution from #7 to #1 (reverse order of serial dilution), samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature or Optional incubate for 14 hours or overnight at 2 ~ 8 °C.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 90 minutes on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 45 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for 15 minutes on microplate shaker at room temperature. **Protect from light.**
10. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well within 3 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log or 4-parameter curve fit to more accurately quantify the standard dilutions.
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL STANDARD CURVE

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450 NM (CORRECTED*)
Blank	0 (0.102)
500	0.021
1000	0.042
2000	0.079
4000	0.129
8000	0.269
16000	0.564
32000	1.209
Optional 64000	1.599

Human FAM19A1/TAFA1 ELISA Kit

Catalog No.: SK00419-01 Size: 96T, 192T

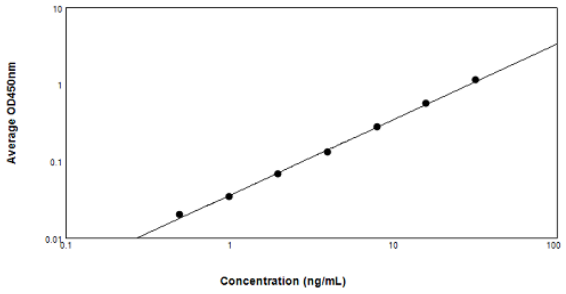
Assay Range: 0.5 ~ 32 ng/mL

Sensitivity: 100 pg/mL

Calibration: rh FAM19A1

Sample Type: Serum, EDTA Plasma, CSF

Aviscera Bioscience manufacture FAM19A1 ELISA



SPECIFICITY

PROTEIN	CROSS-REACTIVITY (%)
Human FAM19A1 His Tag	100
Human FAM19A5 His Tag	0
Human FAM19A2 His Tag	0
Human FAM19A4-Fc (HEK293)	0
Human FAM19A2-Fc (HEK293)	0

Rat and mouse serum or EDTA plasma samples can be detected by this ELISA Kit.

Aviscera Bioscience Manufactures Neuropeptides and new Neurokines Immunoassay Kits:

- Beta Amyloid (1-42) Human ELISA Kit SK00756-06
- Beta Amyloid (1-40) Human ELISA Kit SK00755-06
- CHGA (19-131) Human ELISA Kit SK00084-02
- CHGA (19-94) Human ELISA Kit SK00084-09
- BDNF Human ELISA Kit SK00752-01
- Pro BDNF Human ELISA Kit SK00752-09
- Soluble Sortilin Human ELISA Kit SK00472-01
- Soluble Neprilysin Human ELISA Sk00742-09
- ATP6AP2/Pro-Renin Receptor Human ELISA Kit SK00274-11
- Soluble ACE2 Human ELISA Kit SK00707-06
- Pro NGF (19-121) Human ELISA Kit SK00307-08
- Soluble CSF1R Human ELISA Kit Sk00144-06
- Soluble TREM2 ELISA Kit SK00218-12A
- Neuronal Cell Adhesion Molecule (NrCAM) (Human) ELISA Kit SK00266-01

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to the well. Incubate 2 hours on plate shaker at RT. Optional incubate for overnight at 2 ~ 8 °C.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 90 min on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 45 min on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 15 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 3 min.

Aviscera Bioscience Manufactures Neuropeptides and new Neurokines Immunoassay Kits:

- Cerebellin-1 (Human) ELISA Kit SK00361-01
- Neuronal Cell Adhesion Molecule (NrCAM) (Human) ELISA Kit
- Serum Amyloid A-4 Protein (SAA4) (Human) ELISA Kit SK00778-01
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